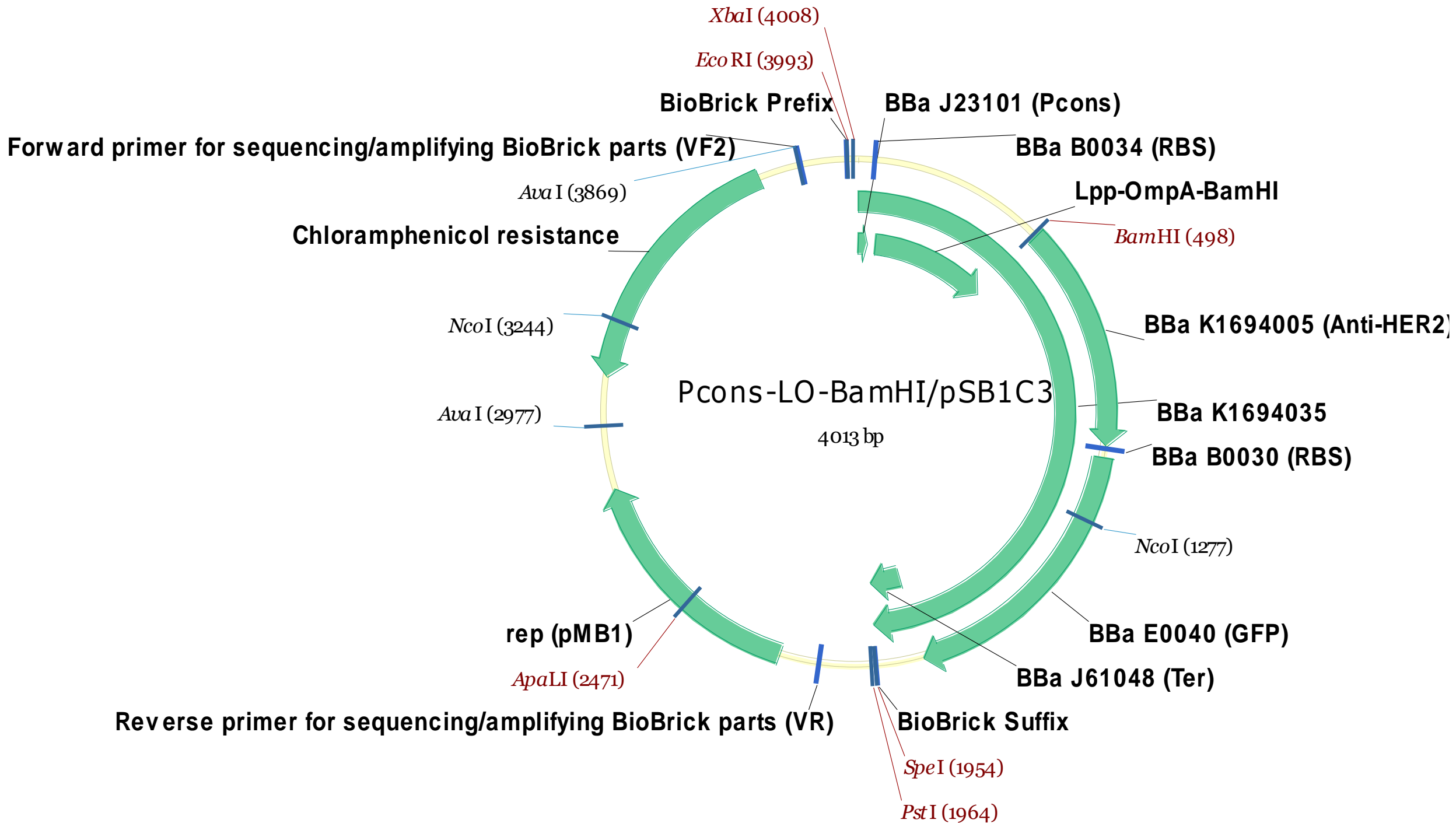


**Pcons-RBS-LO-
BamHI/pSB1C3**



Strategy

- Vector: pSB1C3 (2070 bp) → EcoRI + SpeI
- Insert: EcoRI-XbaI-Pcons-RBS-Lpp-OmpA-BamHI-SpeI amplified by PCR → EcoRI + SpeI
 - Template: NCTU #3_BBak_K1694035_pSB1C3 (#3) Pcons-RBS-Lpp-OmpA-NcoI/pSB1C3
 - PCR product size: 531 bp

Primer Design

- Pcons-XbaI-EcoRI-F:

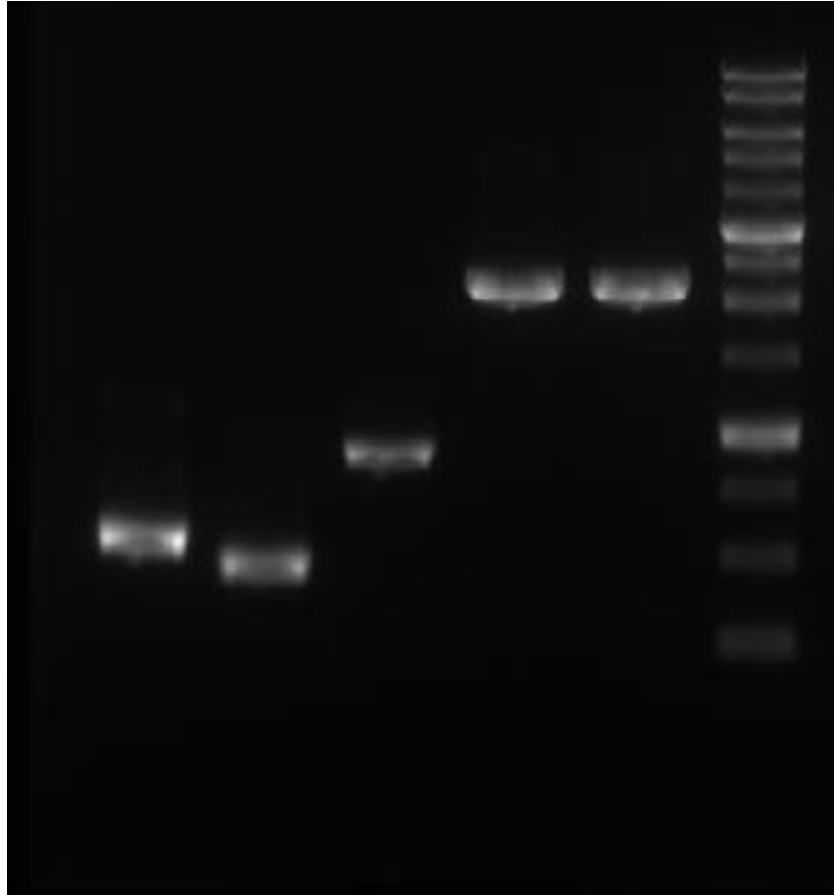
- 5'-

- AGTACAGAATTCGCGGCCGCTTCTAGAGTTTACAGCTAGCTCAG -
3'

- OmpA-BamHI-SpeI-R:

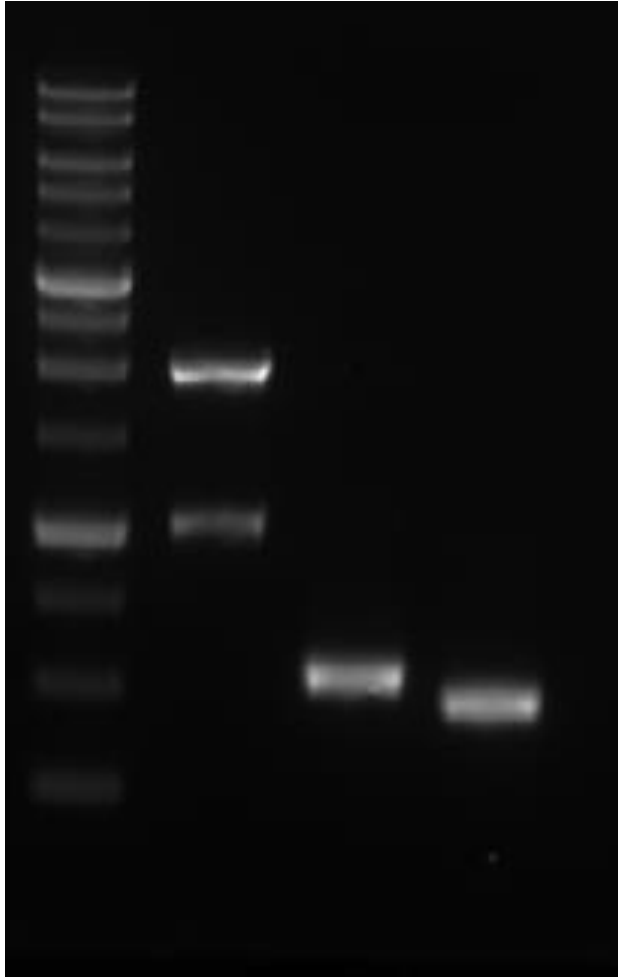
- 5'- AAAGAGACTAGTAGGATCCACGTGTGGCAATTTCGGGGTGA
-3'

PCR with KOD-plus



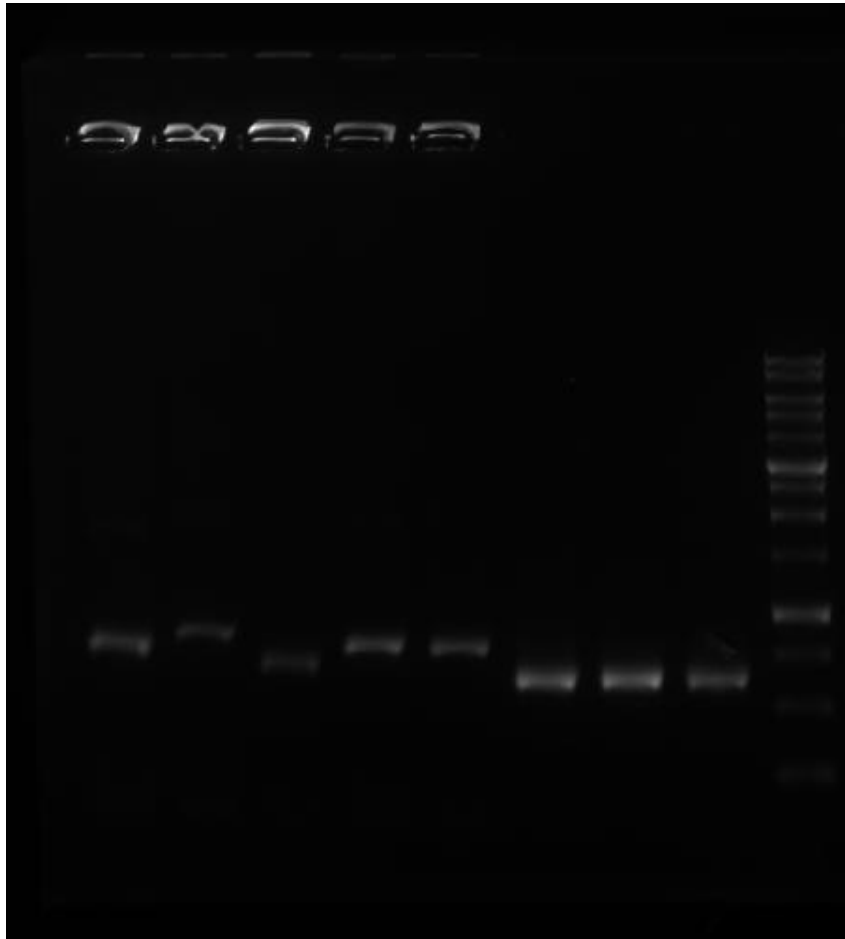
- Date: 2016.08.17
- Lane:
 1. Pcons-RBS-LO-BamHI
 2. LO-BamHI
 3. GFP
 4. AOX1
 5. AOX2

Ligation



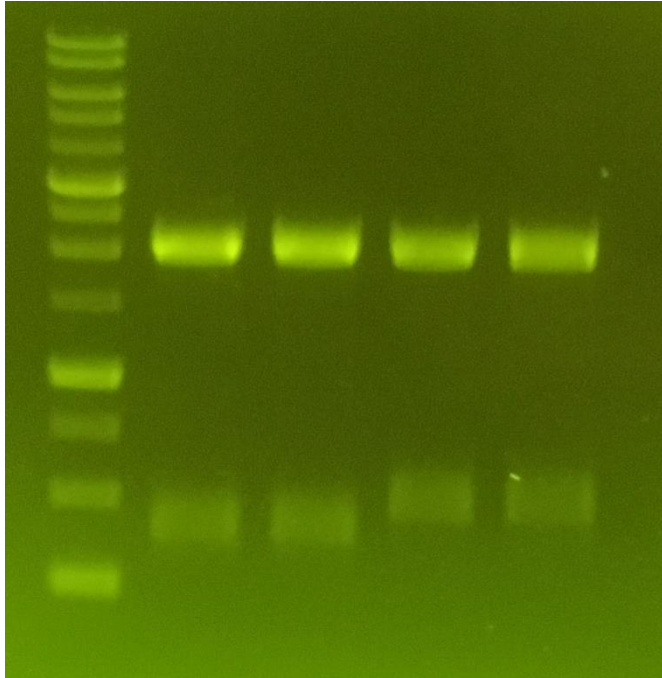
- Date: 2016.08.18
- Lane:
 1. pSB1C3: EcoRI + SpeI
 2. Pcons-RBS-LO-BamHI: EcoRI + SpeI
 3. LO-BamHI: EcoRI + SpeI

Colony PCR



- Date: 2016.08.19
- Clone: Pcons-RBS-LO-BamHI/pSB1C3
- Primer:
 1. VF2
 2. LO-BamHI-SpeI-R
- Lane: #P1 ~ #P8
- #P6, 7 → Plasmid extraction → RE check

RE check



- Date: 2016.08.23
- Clone: Pcons-RBS-LO-BamHI/pSB1C3
- RE: EcoRI + PstI
- Lane:
 1. #L2 (LO-BamHI/pSB1C3)
 2. #L3 (LO-BamHI/pSB1C3)
 3. #P6
 4. #P7
- #P6 → Sequencing

Sequencing

- Date: 2016.08.27
- Sequencing primers: VF2
- Clone: Pcons-RBS-LO-BamHI/pSB1C3 #P6
- File: “2016.08.27_Pcons, LO-BamHI_pSB1C3(#P6, #L2)”
- Result: **correct** → make a stock